Quality Assurance/Quality Control Manual

Ohio District Microbiology Laboratory

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Quality Assurance/Quality Control Manual

Ohio District Microbiology Laboratory

ABSTRACT

The U.S. Geological Survey (USGS), Ohio District Microbiology Laboratory (the ODML) provides water-quality data on microorganisms of public health significance for a variety of projects within the USGS. Currently, the ODML analyzes samples for and provides training on bacterial indicators, coliphage, enteric viruses, and two protozoan pathogens—*Cryptosporidium* and *Giardia*.

Quality-assurance and quality-control (QA/QC) practices for the operation of ODML are described in this manual. The Laboratory Manager, Laboratory Coordinator, Chemical Hygiene Officer, and laboratory and field staff are responsible for implementing QA/QC procedures. This includes correctly following methods of analysis, media and reagent preparation and storage, and analytical quality-control procedures. A sample management and documentation system involves the use of service request forms and login ID's for each sample. Laboratory equipment is maintained and calibrated, the results of which are entered into equipment QA/QC logbooks.

INTRODUCTION

The U.S. Geological Survey (USGS), Water Resources Division, Ohio District Microbiology Laboratory (the ODML) mainly provides analytical data for projects within the Ohio District. With the growth of microbiological monitoring within the WRD, however, the ODML has begun analyzing samples for other District programs by request. Samples are collected to determine the presence of microbiological organisms of public health significance in ground waters and surface waters for a variety of study objectives. For example, some local studies are done to judge compliance with standards for protection of public health in swimmable or drinkable waters. Other studies investigate the occurrence, distribution, and trends of pathogenic organisms and indicators in surface and ground waters and relate these to environmental and water-quality factors.

The ODML fulfills analytical requirements of the WRD by analyzing environmental samples for bacterial indicators, coliphage, enteric viruses, and two protozoan pathogens—*Cryptosporidium* and *Giardia*. ODML personnel provide assistance for project planning and training on three major groups of microorganisms of public health significance in the United States: bacteria, protozoa, and viruses. As funds become available for expansion, the ODML plans to add other analytical methods and types of microorganisms to its analytical list. The ODML is not involved in method development at the present time, but instead tests new methods developed by others for applicability to ambient monitoring programs.

The ODML is committed to providing quality microbiological analytical services to the USGS. The quality assurance/quality control (QA/QC) program is designed to ensure the production of scientifically sound, legally defensible data of known and documented quality. The effectiveness of this program relies on clearly defined objectives, well-documented procedures, and management support.

Purpose and Scope

The purpose of this manual is to identify and document practices and standard operating procedures for those activities of the ODML that affect quality of data. The manual provides ODML personnel and customers with general descriptions of quality practices and goals to aid in the interpretation of data. This manual is intended to be an unpublished, dynamic document that will be frequently updated as laboratory activities expand or change.

Organizational Structure

The Laboratory Manager (1) oversees the daily operations of the ODML, (2) directs technical personnel in the proper performance of laboratory procedures and the reporting of results, (3) ensures that appropriate methods are used and equipment is properly maintained and operated, (4) plans activities leading to testing and modification of analytical procedures, and (5) designs and implements a comprehensive QA/QC program. The Laboratory Manager is responsible for initiating the QA/QC program, providing information and training to the staff, and periodically reviewing QA/QC activities.

The Chemical Hygiene Officer oversees safety operations in the laboratory with assistance from the Laboratory Manager and Laboratory Coordinator.

The Laboratory Coordinator orders supplies and equipment, maintains and calibrates laboratory equipment, and oversees and performs analytical work. The Laboratory Coordinator implements the QA/QC program in the daily tasks of conducting analyses, performing quality control checks, and calculating and reporting results.

The laboratory and field staffs are responsible for correctly implementing collection and analysis procedures and for identifying and working with supervisors to correct and avoid potential problems.

Table 1. Current laboratory personnel and qualifications.

NAME	LABORATORY TITLE	USGS TITLE	Education/experience
Donna Francy	Laboratory Manager	Hydrologist GS-13	B.A. Biology, M.S. Environmental Science, Certified Clinical Microbiologist 12 years experience in water quality and environmental microbiology
Rebecca Bushon	Laboratory Coordinator	Hydrologist GS-11	B.S. Biology 5 years experience in microbiology
Stephanie Janosy	Chemical Hygiene Officer	Hydrologist GS-11	B.S. Zoology M.S. Environmental Assessment Received hazardous material training
Don Stoeckel	Laboratory Staff	Hydrologist GS-11	B.S. Microbiology M.S. Environmental Science Ph.D. Soil Science 4 years experience in microbiology
Chris Kephart	Laboratory Staff	StuTrain(Hyd) GS-7	B.S. Microbiology 3 years experience in microbiology
Amie Gifford	Laboratory Staff	Hydrologist GS-7	B. S. Environmental Science B.S. Plant Biology M.S. Environmental Science 3 years experience in microbiology
Emma Granger	Laboratory Staff	Hydrologist GS-5	B.S. Microbiology 1 year experience in microbiology
Kim Mauch	Laboratory Staff	Hydrologic Technician GS-5	Undergraduate student 1 year experience in microbiology

GENERAL LABORATORY QUALITY ASSURANCE/QUALITY-CONTROL PRACTICES

An overview of analytical methods, training policies, safety, laboratory maintenance, sample management, and data documentation is given in this section.

Analytical methods

The methods used by the ODML can be categorized into four groups: compliance, official, provisional, and experimental. The United States Environmental Protection Agency (USEPA) and others in the research community are continuously developing new methods for detecting and quantifying microbiological pathogens and indicators in water; therefore, several types of methods for target organisms may be currently in use at the ODML.

Compliance methods are those published by USEPA in the Federal Register and are used to determine compliance with standards for protection of public health in swimmable or drinkable waters. Analytical methods for fecal-indicator bacteria are often in this group because they are straightforward, quantitative, and routinely used.

Official methods are those noncompliance methods published by water-analysis authorities such as American Public Health Association, the U.S. Environmental Protection Agency, or the USGS. Official methods should be well established, have known levels of bias and variability, and be relatively easy to apply in field operations or have holding times long enough to allow shipping to a central laboratory for analysis.

Provisional methods are published methods that are still being validated by the method developer, usually the USEPA. For these methods, the method developer establishes precision and accuracy and ensures the methods are adequately tested. Because methods for detection of protozoa are complex, qualitative to semiquantitative, expensive, and very time consuming, these methods are often provisional.

Experimental methods are unpublished methods that are currently being testing to establish QA/QC practices and determine applicability to ambient monitoring programs.

Training

The Laboratory Manager and Laboratory Coordinator are responsible for ensuring that laboratory employees receive proper training in analytical methods and laboratory procedures and for documenting any training received. In particular, laboratory employees will be trained in sterile technique before handling samples for microbiological analysis. A new employee will receive orientation and skills training. New or established employees may receive training on new methods given by the method developer. The Laboratory Coordinator will maintain training records for microbiological methods on file by employee; this includes on-the-job training as certification of proficiency in microbiology.

The Laboratory Manager, Chemical Hygiene Officer, and District Safety Officer provide safety orientation to new employees and safety education to all employees. The employee orientation covers general safety issues, emergency procedures, standard-safety operations, the chemical-hygiene plan, hazardous-waste management, waste disposal, and location of safety equipment.

Safety

Detailed laboratory safety practices and responsibilities are described in the Chemical Hygiene Plan. Safety activities include safeguards to avoid electric shock; prevent fire; prevent accidental chemical spills; and minimize microbiological dangers, facility deficiencies, and equipment failures.

Laboratory personnel that are isolating microorganisms from natural sources must be made aware that pathogens may be present in environmental samples. Technicians are to wear disposable gloves and lab coats when handling samples that are likely to contain pathogens. Safety glasses are worn if there is a chance of projectiles, aerosols, or other foreign matter entering the eye. This includes when using positive-pressure air to blow out any remaining liquid during the ultrafiltration process for *Cryptosporidium* and *Giardia.* Laboratory personnel will receive immunizations for pathogens on a project-specific basis. Each project sending samples to the ODML is required to have a project safety plan--copies are available for ODML employees. Immunizations are offered to all ODML workers for Hepatitis A virus, Hepatitis B virus, and tetanus.

Safety equipment is tested at regular intervals. Safety showers, eyewashes, and fire extinguishers are tested annually. The Chemical Hygiene Officer maintains a list of chemicals and arranges for a contract for disposal of hazardous waste.

Laboratory materials and equipment

The Laboratory Manager sets policies for preventive maintenance and calibration of laboratory materials and equipment. Three equipment QA/QC logbooks are kept in the laboratory bookshelf with records of quality-assurance checks of materials and equipment. The logbooks are for (1) autoclaves, balances, pipettors, hoods, and the vacuum pump; (2) laboratory water, and (3) incubators, water baths, refrigerators, and freezers. Examples of equipment log sheets are in Appendix AA. Quality-control checks that are required equipment QA/QC logbook entries are listed in italics below.

- ***The Laboratory Manager or Laboratory Coordinator must review all entries and sign off each page of the equipment QA/QC logbook quarterly to ensure procedures are followed and problems are properly addressed.***

For some pieces of equipment, the use of daily logbooks to record operating times and other types of frequent entries is required. A daily logbook is kept with the autoclaves the vacuum pump, and the water-quality meters (pH, specific conductance, and turbidity).

General sterility and cleanliness

The sterility and cleanliness of the laboratory is necessary to ensure the integrity of samples and analytical procedures.

- **Traffic through the laboratory is restricted to those doing work in the laboratory, especially when analytical work is being done.**

- **The countertops are wiped down with surface disinfectants, such as Envirocide (Metrex Research Corp., Romulus, MI) or 70 percent ethanol, before and after use.**

- **Antimicrobial soap is available at various laboratory sinks to facilitate hand washing before and after laboratory work.**

Clean and sterile glassware that is free of detergent residue is crucial to ensure valid results in microbiology.

- **Dirty dishes are placed on a moveable laboratory cart after use and are not to be stored on countertops. Dishes are washed in a dishwasher or by hand with hot water and laboratory-grade phosphate-free detergent. Dishes are rinsed with tap water and then deionized water.**

Autoclaves

Sterilization is the process that eliminates living organisms from substances or objects. The ODML is equipped with three autoclaves for sterilization of glassware, reagents, media, and disposables—two medium-sized autoclaves (Market Forge) that are operated in the main laboratory and one large autoclave (Consolidated) that is operated in the warehouse.

- **Dishes that need to be sterilized are wrapped in aluminum foil or kraft paper and placed in the autoclave for moist heat sterilization. Clean and sterile dishes are stored in closed cupboards until use.**

- **The autoclaves are operated at 15 lb/in^2. steam pressure, producing an inside temperature of 121 to 124^{o}C (American Public Health Association, 1998, Section 9020B). Do not overload the autoclave. Autoclave time depends on the type and amount of equipment as follows:**

  - **Glassware and up to 250 mL of liquid—15 minutes**

  - **500 to 2,000 mL liquid—30 minutes**

  - **Greater than 2,000 mL to 6,000 mL liquid—15 minutes per 1,000 mL**

  - **Greater than 6,000 mL liquid—90 minutes**

  - **Carbohydrate-containing media—15 minutes (no more than 250 mL volumes)**

  - **Contaminated materials and discarded cultures—45 to 90 minutes**

- **The time of each run is recorded in daily autoclave logbooks, kept near the autoclaves. Operating temperature and pressure are checked periodically. Heat-sterilizing tape is used with each run to identify supplies that have been properly sterilized and checks the performance of the autoclave. *The performance is also checked quarterly by using spore indicators and recorded in the equipment QA/QC logbook.***

- **If the autoclave does not reach the specified temperature or fails the spore indicator test, service the autoclave and re-sterilize all glassware and reagents that were insufficiently sterilized.**

For the two medium-sized autoclaves, general maintenance is as follows:

- **The autoclaves are operated using deionized water.**

- **At the end of the day, autoclaves are drained. Twice a month, autoclaves are cleaned with mild soap, rinsed with water, and drained. The condensate holding**

tank is drained daily or as needed. The cleaning date is recorded in the daily autoclave log book.

- **Twice a year, have a contractor clean, inspect, and calibrate the autoclaves and perform preventive maintenance.**

For the large autoclave, general maintenance is as follows:

- **Manually drain the generator the day after the autoclave is used, while the autoclave is turned off. Leave valve open for 15 minutes.**

- **Once a month, clean chamber with water and liquinox.**

- **Twice a year, have a contractor perform preventive maintenance and inspection, clean and service the generator, clean the door gasket and head ring, apply graphite to the door gasket, oil the door hinge pins, and lubricate the door hub.**

Laboratory water

The ODML has three types of laboratory water:

(1) Type III deionized water ("deionized water") produced from City of Columbus tap water for general laboratory use. The deionized water unit and tap are stored in the warehouse. The system is described in Francy and others (1998). Every quarter, the vendor changes the cation and anion columns, moves forward the standby mixed-bed column, installs a new standby tank, and changes the carbon filter. The 5-µm pore-size prefilter is changed twice a year. ***Maintenance checks are recorded in the equipment QA/QC log book*****.**

(2) Reagent-grade water produced using a Millipore MilliQ system ("MilliQ water"). Deionized water is used as source water for the MilliQ system. Reagent water is used for cultivation media and additives (mTEC, MI, mEI, antibiotic stocks, and others) as well as for preparation of reagents for sensitive procedures (elutions, PCR, hybridization, and others). The MilliQ cartridges are changed by ODML laboratory personnel when the service light blinks and the display message reads "EXCH. CARTRIDGES." ***Indicate the date of cartridge change in the equipment QA/QC logbook.***

(3) Deionized water stored in a laboratory carboy ("stored water") and used for rinsing of dishware, cleaning the autoclaves, and rinsing of other supplies.

A variety of quality-control checks are routinely done on the three types of water and may differ depending on the type of water. Acceptance criteria are listed in table 2. For deionized water, two levels of acceptance criteria are listed—(1) a warning level wherein the system is inspected and constituents are retested and (2) a

shut-down level. For MilliQ water, only a shut-down level is listed in table 2. For stored water, if criteria are not met, the container is cleaned out and refilled.

- ***Quarterly checks of specific conductance and turbidity are done on all three types of water and recorded in logbook.*** **Instructions for performing these checks are in the back of the equipment QA/QC logbook.**

- ***Quarterly checks of bacterial growth are done on the MilliQ water and recorded in the equipment logbook.*** **Instructions for performing this check are in the back of the equipment QA/QC logbook.**

- ***A blank of deionized water is submitted to the National Water Quality Laboratory (NWQL) annually and analyzed for low level nutrients (Schedule 1217), and total-organic carbon (Labcode 114).*** **We no longer analyze a blank for trace elements and low-level major ions because the need for these low-level analyses is project specific.**

- **The stored deionized water carboy is to be emptied completely and cleaned every other week.** ***Record cleanings in the equipment QA/QC logbook.***

Table 2. Acceptance criteria for laboratory water quality-assurance checks [Adopted from USEPA (1978), APHA (1998), and ASTM (1999); NA is not applicable; constituents highlighted in gray are no longer required tests]

	DEIONIZED		MILLIQ	STORED
ACTION	warning	shut down	shut down	clean and refill
Specific conductance (μs/cm)	3	5	2	3
Turbidity	1	5	1	1
Heterotrophic plate count (colonies/mL)	NA	NA	<1	NA
Total organic carbon (mg/L)	0.2	10	NA	NA
Sodium (mg/L)	0.1	1	NA	NA
Nutrients individual (mg/L)	0.1	1	NA	NA
Heavy metals, individual (Cd, Cr, Cu, Ni, Pb, Zn) (μg/L)	1	10	NA	NA
Other trace elements (μg/L)	3	50	NA	NA

Analytical balances

Analytical balances are used for accurate weighing of reagents and media. *They are checked and calibrated annually by the manufacturer's service technician, and the results are recorded in the equipment QA/QC logbook*. Balances must rest on a firm, level surface. Balance trays are wiped off daily with water or a surface disinfectant, such as Envirocide or 70 percent ethanol.

Hoods

The Ohio District has four types of hoods— (1) a biosafety cabinet, (2) a laminar-flow hood, (3) a hazardous-waste fume hood, and (4) a PCR workstation. To ensure proper use of hoods 1, 2, and 3, "quick check" criteria for properly running each hood are posted on the hoods.

- ***The operation of hoods 1, 2, and 3 are checked and certified by a qualified inspector annually and recorded in the equipment QA/QC logbook*.**

The biosafety and laminar flow hoods have magnehelic pressure gauges (MAG) that are used to monitor operation of the hoods. When using either hood, check to make sure the pressure gauge is reading at a level approximately equal to the annually recorded MAG level on the calibration sticker. A significant increase in pressure indicates that the filters are dirty whereas a significant decrease in pressure indicates an electrical problem.

The biosafety cabinet, laminar-flow hood, and PCR workstation (Hoods 1, 2, and 4) must be free from contamination by live organisms.

- **The working surfaces of the laminar-flow hood, the biosafety cabinet, and the PCR workstation (Hoods 1, 2, and 4) are wiped down with a surface disinfectant, such as Envirocide or 70 percent ethanol before and after use. For the biosafety cabinet, be sure to lift up the work surface and clean under this area periodically.**

- **The biosafety cabinet and PCR workstation (Hoods 1 and 4) have ultraviolet bulbs for germicidal purposes. The ultraviolet lights in the biosafety cabinet and PCR workstation are cleaned quarterly by wiping the bulbs with a soft cloth. *Record cleaning date in the logbook.* A bulb that is dull in the center needs to be replaced. *Record the bulb change in the equipment equipment QA/QC log book.***

- **Biannually, nonselective agar plates are exposed to airflow in the laminar-flow hood, the biosafety cabinet, and PCR workstation for 1 hour (Hoods 1, 2, and 4). The plates are incubated at 35^{o}C for 24 hours and examined for contamination. *The results are record in the equipment QA/QC logbook.***

The hazardous-waste fume hood (Hood 3) must be checked to ensure that it is operating properly.

- ***Check the operation of the hazardous-waste fume hood (Hood 3) quarterly by use of fume cartridges and record results in the logbook*.**

Specific conductance, pH, and turbidity meters

With each use of the specific conductance, pH, or turbidity meter, calibrate the instrument according to the manufacturer's instructions (kept with the meter). Use a calibrated solution that is within the range of the water sample to be measured. Label specific conductance and pH buffer solutions with the date opened and discard working solution weekly. Each piece of equipment has daily logbook; record all calibrations in the appropriate logbook.

Micropipettors

Micropipettors are used for the accurate delivery of small volumes.

- **Pipettors are sent to the manufacturer annually for cleaning, preventative maintenance, calibration, and adjustment, if necessary. *The maintenance dates are recorded in the logbook.* Preventative maintenance includes a new seal and piston cleaning annually, and a new shaft and reconditioned piston every 3 years.**

Vacuum pump

The vacuum pump is mainly used for membrane filtration. The time of each run (at least 15 minutes) is recorded in a logbook kept with the vacuum pump. After 500 hours of use, the oil is changed. *Record the oil change in the equipment logbook.*

Incubators, water baths, refrigerators, freezers, and thermometers

There are 3 incubators, 4 water baths, 3 refrigerators and 5 freezers in use in the laboratory. Temperature settings and criteria for acceptance are dependant on use (table 3). Approximately 11 aluminum-block blue field incubators and 2 double-chamber gray incubators are used for laboratory and field operations.

- ***The temperatures of the laboratory incubators, water baths, refrigerators, and freezers are checked quarterly with laboratory thermometers.***

- ***The operating temperatures of microbiological aluminum-block incubators are checked annually (or in preparation for a major study) and recorded on the outside of each incubator and in the equipment QA/QC log book*. During period of heavy use, the temperatures are checked and recorded weekly.**

- **The two –70°C freezers (freezers 3 and 4) are used to store samples and microbiological cultures.** ***A filter is cleaned and fans behind the filter are checked by laboratory personnel for operation quarterly. The condenser is dusted or vacuumed every 6 months. A temperature chart is changed after a single pass around the chart (weekly).***

- **Water baths are filled with distilled water only and are cleaned with mild soap quarterly, or more often as needed.** ***Record quarterly cleanings in the equipment Equipment QA/QC logbook.***

Table 3. Acceptance criteria for laboratory refrigerators, freezers, incubators, and waterbaths

Equipment identification	Use	Acceptance criteria
INC 1	Method 1601/1602, transfer cultures	36°C ± 1.0°C
INC 2	*Actinomycetes, Clostridium*	28°C ± 1.0°C, 42°C ± 0.5°C
INC 3	Hybridization oven	51°C ± 1.0°C, 80°C ± 1.0°C
INC 4	Fungi, general use	36°C ± 1°C
W/B 1	Melt and temper agar	48°C ± 3.0°C
W/B 2	Method 1602, thaw hosts	37°C ± 1.0°C, 48°C ± 3.0°C
W/B 3	Grow hosts for Method 1601/ 1602	36°C ± 1.0°C
W/B 4	Shaking bath for hybridization	51°C ± 0.2°C
REFRIG 1	Sample storage	1 to 4°C
REFRIG 2	Reagent/ media storage	1 to 4°C
REFRIG 3	Reagent storage	1 to 4°C
FREEZ 1	PCR reagents	-20°C to -30°C
FREEZ 2	Ice packs, reagent storage	-20°C to -30°C
FREEZ 3	Host stocks, sewage filtrate, virus stocks, samples	Shelf 1 -70°C ± 10°C Shelf 2 -70°C ± 10°C Shelf 3 -70°C ± 10°C Shelf 4 -50°C ± 10°C Shelf 5 -50°C ± 10°C
FREEZ 4	Sample storage	-70°C ± 10°C
FREEZ 5	Probes, hybridization reagents	-20°C to -30°C
FIELD INCUBATORS	Membrane filtration	35°C ± 1.0°C, 44.5°C ± 0.5°C

Thermometers are kept in three areas and are inventoried according to storage and use: (1) extra thermometers for general laboratory use, including the National

Institute of Standards and Technology (NIST) thermometer (2) water-bath thermometers, (3) digital thermometers, and (4) back-lab thermometers.

- **The NIST thermometer is calibrated and certified annually by an outside service technician.** ***Certification reports are kept in the equipment QA/QC logbook.***

- ***Laboratory thermometers are checked biannually against the NIST thermometer. Results are recorded in the equipment QA/QC logbook and acceptance criteria are listed in table 4.*** **Criteria are based on use.**

Table 4. Acceptance criteria for laboratory thermometers

Thermometer use	Thermometer identification (will vary)	Location	Test Temperature	Criteria
Extras	NIST	Drawer	Certified	Certified
		Drawer	36°C	± 1°C
Water bath		W/B 1	48°C	± 1°C
		W/B 3	36°C	± 0.5°C
Digital		Drawer	30 °C and 48°C	± 0.1°C
Back lab		Freezer 5, back lab	30°C and 0 °C	± 1°C
		W/B 4, back lab	51°C	± 0.1°C

Microscope and Centrifuge

The microscope is used for general laboratory work and for enumerating *Cryptosporidium* oocysts and *Giardia* cysts by USEPA Method 1623. For Method 1623 the microscope is equipped with excitation/bank-pass filters for immunofluorescence assay (FA) and 4', 6-diamidino-2-pheylindole (DAPI), and stage and ocular micrometers. The microscope is kept in a room capable of being darkened to near-complete darkness.

- ***The date and time the mercury bulb is turned on and off is entered in the microscope log book*. After 125 hours, the mercury bulb must be changed. Record the mercury bulb number and date of installation in the equipment log book.**

- **The microscope is cleaned and the ocular micrometer is calibrated yearly by the manufacturer. *Record this maintenance in the equipment log book*.**

Each run of the centrifuge is recorded in the equipment log book. The temperature is monitored quarterly with the digital thermometer (acceptance criteria is 4± 3ºC). The buckets are cleaned with soap and water quarterly. Lubrication is done annually, or as needed. *All maintenance is recorded in the equipment log book*.

Sample Management and Documentation

Samples for the bacterial indicators, *E. coli*, enterococci, fecal coliform, and total coliforms, are most commonly processed and analyzed in the field; however, they may be done in the ODML. Holding times are 6 hours for compliance purposes and 24 hours for noncompliance purposes (American Public Health Association, 1998, Section 9060 B.) Adhering to a 6-hour holding time for all bacteriological samples, however, is highly recommended.

Samples for *Clostridium perfringens*, coliphage, and enteric viruses are processed and analyzed in the ODML; samples are kept on ice and processed within 48 hours of sample collection. Samples that arrive chilled within 48 to 96 hours from collection are acceptable, but the results are qualified. Samples for *Cryptosporidium* and *Giardia* are processed in the ODML within 48 hours of sample collection and analyzed in a contract laboratory. Although the samples for *Cryptosporidium* and *Giardia* are not kept on ice during transport, they are stored in the refrigerator upon receipt in the ODML.

All requests for laboratory analysis must be submitted using a service request form (Appendix A). The following categories must be filled out when requesting sample analysis: station name and date/time of sample collection. The field personnel must check off requested analyses unless prior arrangements with laboratory personnel have been made. Upon receipt of the sample in the laboratory, personnel will fill in Received By and Date Received on the front of the Service Request form.

Laboratory personnel will then enter sample information on a log sheet (Appendix B) and assign a login ID. There are five logbooks as follows: (1) bacterial indicators (whole-water samples), (2) coliphage (whole-water samples), (3) enteric viruses (water samples concentrated on filters by field personnel), (4) Actinomycetes (whole-water samples), and (5) *Cryptosporidium* and *Giardia* (whole-water samples in cubitainers). Different login ID's are assigned to these five groups of microorganisms. Laboratory personnel will write the login ID on the Service Request form (front and back) and on the sample bottle (or filter cartridge).

The Service Request form is routed to the analyst, who will enter sample login ID, processing times, and analytical information on a separate Results Worksheet (Appendix A). Samples are analyzed within 24 hours of receipt and are stored in the laboratory refrigerators until processing.

Upon completion of the analysis, the analyst writes final results on the back of the Service Request form. A second analyst routinely checks the calculations of the analyst performing the work. The results are entered into a spreadsheet and then transmitted to the person requesting the analysis by email. The Service Request form and Results Worksheet are then filed together in the appropriate logbook.

METHODS OF ANALYSIS, MEDIA AND REAGENT PREPARATION, AND ANALYTICAL QUALITY-CONTROL PROCEDURES

Methods of analysis, media and reagent preparation and storage, and analytical quality-control procedures are discussed in this section. Because microbiological analyses measure constantly changing living organisms, the methods are inherently variable. Some quality-control tools used by chemists, therefore, may not be available to the microbiologist (American Public Health Association, 1998, Section 9020 A).

References of published microbiological methods are kept in a notebook in the laboratory. Media-preparation instructions and method summaries written by the ODML are kept in the reference notebook and furnished as Appendixes to this document.

Fecal-indicator bacteria

The methods used for analysis of fecal-indicator bacteria are those of the USGS, USEPA, and APHA and others (table 5). All fecal-indicator bacteria methods used by the ODML are compliance or official methods.

Table 5. Methods for fecal-indicator bacteria analysis used by the Ohio District Microbiology Laboratory (ODML) [DW is drinking water, RW is recreational water]

BACTERIA	METHOD	TYPE OF METHOD	REFERENCE
Total coliforms	mENDO method	Compliance—DW Official—other waters	Britton and Greeson (1987) APHA (1998) Section 9222B
	MI method	Official—all waters	USEPA (2000a) (Appendix C)
	Colilert method	Compliance—DW Official—other waters	Idexx Corp., Westbrook, ME APHA (1998) Section 9223 (Appendix D, E)
Fecal coliforms	mFC method	Compliance—DW Official—other waters	Britton and Greeson (1987) APHA (1998) Section 9222D
Escherichia coli	mTEC method	Compliance—RW, DW	USEPA (1985) (Appendix F)
	MI method	Official—all waters	Brenner and others (1993)
	Colilert method	Compliance—DW Official—other waters	Idexx Corp., Westbrook, ME APHA (1998) Section 9223
	Modified mTEC	Official	USEPA (2000b) (Appendix G)
Enterococci	mEI method	Compliance—RW Official—other waters	USEPA (1997) (Appendix H)
Clostridium perfringens	Modified mCP method	Official	USEPA (1996), modified by ODML (Appendix I)

Reagents and media for fecal-indicator analysis are prepared according to the methods and are labeled to indicate date prepared. Each lot of media and buffered-dilution water is quality-control tested by using a pure culture of the target bacterium or a sewage sample as a positive control; for modified mTEC, Colilert, and MI agars, negative controls are also required (Appendix J). Fresh sewage samples are obtained from the Olentangy Wastewater Treatment Plan weekly, as needed. Stock cultures of the positive and negative controls are kept on slants in the refrigerator and transferred once a month. When preparing positive and negative controls to be sent to other Districts, stock cultures are transferred on Monday for preparation on Tuesday, Wednesday, or Thursday. *Transfers are recorded on QA/QC stock culture log sheets.*

Results are recorded in the "Media and Buffer" logbook on quality-control sheets (Appendix K); documentation of preparation procedures is also kept in this logbook. Media storage requirements and holding times are strictly followed (table 6). Requests for media, buffered-dilution water, and reagent preparation by project personnel are made using the "Expendable supplies request forms" (Appendix L).

The type of buffered-dilution water used most often by the ODML is phosphate buffered dilution water (U.S. EPA, 2000b), labeled with purple tape. Instructions for preparation are listed in Appendix M. Two other types of buffer, FC buffer with peptone and EC buffer, are no longer routinely used by the ODML but are kept in Appendix M for historical purposes.

Table 6. Information on media, buffered-dilution water, and reagents prepared and stored in the Ohio District Microbiology Laboratory (ODML). [QWSU is the Quality of Water Service Unit in Ocala, Florida]

TYPE OF MEDIA/BUFFER	SOURCE	STORAGE	HOLDING TIME
mENDO agar	QWSU	Desiccator	Expiration date for agar kits 3 days as plates
MI agar	ODML	Refrigerator	6 months in dilution bottles 2 weeks as plates
MI agar	S&S Bioscience, West Palm, FL	Refrigerator	45 days from receipt
Colilert	Idexx Corp., Westbrook, ME	Cabinet	Expiration date per manufacturer
mFC agar	QWSU Difco, Detroit, MI	Desiccator Cabinet	Expiration date 3 days as plates
mTEC agar	ODML only*	Refrigerator	6 months in dilution bottles 2 weeks as plates
Modified mTEC	ODML	Refrigerator	6 months in dilution bottles 2 weeks as plates
Modified mTEC	Hach Co., Loveland, CO	Refrigerator	As listed by manufacturer
mEI	ODML QWSU	Refrigerator Desiccator	6 months Expiration date for agar kits 3 days to 2 weeks as plates**
mCP agar	ODML	Refrigerator	6 months in dilution bottles 1 month as plates
PB water (purple tape; Appendix M)	ODML QWSU	Cabinet (unopened) Refrigerator (after opening)	1 year (unopened) 2 weeks (after opening)
Urea-phenol solution (Appendix F)	ODML	Refrigerator	6 months or until it is no longer a straw-yellow color

*** Because of degradation problems with the commercially available dehydrated media, only mTEC agar prepared by the ODML is to be used.**
**** If reagents that are added after autoclaving are filter sterilized, the longer holding time is applied.**

Analytical quality-control samples for fecal-indicator bacteria by membrane filtration (mENDO, MI, mFC, mTEC, modified mTEC, mEI, and mCP agar methods) include the following:

- **Filter blank—a 50-100 mL aliquot of sterile buffered water is plated before the sample to confirm the sterility of equipment and supplies.**

- **Procedure blank—a 50-100 mL aliquot of sterile buffered water is plated after every fifth sample to measure the effectiveness of the analyst's rinsing technique or presence of incidental contamination of the buffered water.**

- **A sewage sample is plated daily when *C. perfringens* analysis is done to evaluate the test procedure and to ensure anaerobic culture conditions.**

- **For MI, positive and negative controls are plated every 10 samples to ensure proficiency with the method and evaluate the integrity of the medium. Positive and negative controls include the following:**

  - **Positive control of *E. coli***
  - **A negative control of *Pseudmonas* ATCC 10145, that is unable to grow on MI and ensures the selectively of the agar**

  For some projects, a sewage sample is plated with each batch of MI plates at the time of sample analysis to evaluate the effectiveness of cefsulodin (an antibiotic added at the time of plate preparation).

Analytical quality-control samples for Colilert include the following:

- **Positive (*E. coli*) and negative-control (*Pseudomonas*) cultures are included with every 20th sample to evaluate the test procedure and aid in interpretation of results.**

Enteric viruses

The reverse-transcriptase, polymerase chain reaction (RT-PCR) and cell-culture methods are recommended for detection of enteric viruses in water. To prepare samples for RT-PCR and cell culture, attached viruses are eluted from a 1MDS filter with beef extract (pH 9.5), concentrated using celite (pH 4.0), and eluted with sodium phosphate (pH 9.5). These steps are done in the ODML, and a protocol is included as Appendix N.

The RT-PCR method was written and laboratory tested by USEPA and is experimental (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997). Viruses are isolated from the eluate by ultracentrifugation through a sucrose gradient, and trace contaminants are removed by extraction with a solvent

mixture; these inhibitor-removal steps are done using the facilities of Dr. Darrell Galloway, Department of Microbiology, The Ohio State University. An ODML employee is able to perform all subsequent analytical steps at the ODML laboratory. An aliquot of the concentrate is used for RT-PCR, wherein any target viral RNA is converted to DNA (by reverse transcriptase) and amplified by the polymerase chain reaction. The RT-PCR products confirmed by hybridization; approximately 5 percent of the samples are also analyzed by agarose gel electrophoresis. The enteric viruses detected by use of this method include enterovirus, Hepatitis A, rotavirus, reovirus, and calicivirus (including Norwalk-like virus).

The ODML consists of a 1,000 ft^2 main laboratory and a 300 ft^2 limited-use laboratory. Virus elutions and reaction preparations for RT-PCR are done in the main lab, along with media preparation, membrane filtration, incubation, and culture maintenance. The limited-use lab is the only area in the building in which PCR products are handled. Gel electrophoresis and hybridization are performed in this room. To avoid contamination of incoming samples, staff that have entered the limited-use lab are not allowed to reenter the main lab unless they have showered and changed their clothes. Equipment and supplies are also not to be transferred into the main lab, unless adequate sterilization and decontamination procedures have been followed.

A list describing QC samples for all stages of sample preparation and analysis by the RT-PCR method are listed in Appendix N1. A check list to be used when interpreting all results of RT-PCR analyses is also contained in Appendix N1.

Cell-culture analysis is not done at the ODML; these samples need to be sent to a contract laboratory for analysis. The recommended cell-culture method is an experimental method and was modified from U.S. Environmental Protection Agency (1996) by USEPA (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1999). The sample eluate is added to a monolayer of a continuous cell line derived from African Green monkey kidney cells. Each cell culture is examined microscopically for the appearance of cytopathic effects (CPE) for a total of 14 days; if CPE is not observed in 14 days, a second passage is done. Results are reported as most probable number of infectious units per volume of water. The following QC samples are included in cell-culture analysis:

- **A negative control, containing cells and sodium phosphate buffer, is incubated in a roller bottle with each batch of samples inoculated for the first passage.**

- **A positive control, containing cells, sodium phosphate buffer, and polio virus vaccine, is incubated in a 75 cm^2 flask with each batch of samples inoculated for the first passage and the second passage.**

Media and reagent preparations are done in the ODML or at the contract laboratory and are prepared and stored per method instructions. The contract laboratory is required to strictly follow the QA/QC guidelines listed in the method

documentation (G. Shay Fout, U.S. Environmental Protection Agency, written commun., 1997 and 1999).

Coliphage

The method currently in use for quantitative coliphage analysis by the ODML is the USEPA Method 1602, single-agar layer (SAL) procedure (USEPA, 2001b) (Appendix O). This method is generally most suitable for quantification of coliphage in surface-water samples. Antibiotic-resistant *E. coli* CN-13 (resistant to nalidixic acid) and *E. coli* F-amp (resistant to streptomycin and ampicillin) are used as bacterial hosts for somatic and F-specific coliphage, respectively. Procedures quality-control samples are described in Appendix O. Results are recorded on a QC log form (Appendix P).

The method currently in use for qualitative determination of coliphage in larger sample volumes at the ODML is the USEPA Method 1601, two-step enrichment method (USEPA, 2001a). Sample volumes of either 1 L or 4 L are recommended for detection of coliphage using this method. Because the SAL method is impractical for sample volumes above 100 mL, the two-step enrichment method is often used for ground-water sample analysis. A summary of Method 1601 can be found in Appendix Q. Results from quality-control samples are recorded on a QC log form (Appendix R).

A coliphage sample log book is kept in the laboratory for both Method 1601 and 1602. A second book, coliphage QC log book, contains information on media sterility, quality-control samples, and coliphage stock enumeration results. Information on host culture strains is also found in the coliphage log book and is maintained on cultivation log sheets (Appendix S).

Cryptosporidium and Giardia

USEPA method 1623 (USEPA, 2001c) is the USEPA-recommended method for detection of *Cryptosporidium* oocysts and *Giardia* cysts in water. This provisional method involves filtration, immunomagnetic separation, staining with fluorescent antibody, and microscopic evaluation. At the present time, the ODML has the capability to complete the sample processing steps—filtration and concentration (Appendix T). The ODML is developing the capability for immunomagnetic separation and microscopy.

Actinomycetes

The Actinomycetes are a large group of filamentous gram-positive bacteria that resemble fungi because they produce mycelium and dry spores, called conidia. (Madigan and others, 2000). They are considered nuisance organisms for those in the water industry, as they are one of two types of organisms that impart an earthy-musty odor to waters. The odors are caused by two compounds formed during

normal actinomycete development, geosmin and 2-methylisoborneol (American Public Health Association, 1998).

The method used for isolation of Actinomycetes from water in the ODML is based on a published method (American Public Health Association, 1998) and a method provided by a commercial supplier of Actinomycetes medium (Difco, Detroit Michigan, The Difco Manual, 2001). To ensure isolation of the target organism, two plating methods are used: (1) the double agar layer method, and (2) the spread plate method (Appendix U). Stock cultures of *Streptomycetes albus* are used as positive controls. The stock culture is transferred every two months and transfers are recorded Actinomycetes QA/QC log book.

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